

# Frequency of *Chlamydia trachomatis* in endocervical samples of women referred to Gynecology Hospital in Qazvin-Iran, by PCR-based assay

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## Abstract

**Background:** *Chlamydia trachomatis* (*C. trachomatis*) is the most common bacterial sexually transmitted infection (STI). Genital *C. trachomatis* infection is leading cause of complications e.g pelvic inflammatory infection (PID) tubal factor infertility and abortion.

**Objectives:** The objective of this study was to estimate the Frequency of *Chlamydia trachomatis* infection among symptomatic and asymptomatic women, by PCR-based assay.

**Methods:** This was a cross-sectional study conducted over the period from, May 2012 to February 2013. A total of 240 nonduplicate endocervical samples were taken from married women. Endocervical swabs were collected from women referred to Qazvin Kowsar Gynecology Hospital by training midwife. The specimens were tested for *C. trachomatis* by PCR-based assay for *phagene*.

**Results:** Out of 240 participants women 184 (76.7%) were symptomatic and 56 (23.3%) were asymptomatic cases. The mean age of cases was  $37.1 \pm 0.9$  years. Twenty (8.3%) of 240 samples were diagnosed as *Chlamydia* positive according to PCR results. The prevalence of asymptomatic *C. trachomatis* infections was 6 (10.7%), while 14 (7.6%) symptomatic cases were infected. The chi2 test was not show a significant relationship between positive test result and vaginosis, abortion, infertility, premature birth, low birth weight below 2.5kg

**Conclusion:** The results of this study showed high prevalence of *C. trachomatis* infection among in both symptomatic and asymptomatic women.

Therefore, a screening test for *C. trachomatis* infection is recommended for all women who refer to genitourinary medicine clinic. Screening programs are important for cost effectiveness calculations in complication of *C. trachomatis* infections especially in asymptomatic cases.

**Keywords:** *Chlamydia trachomatis*, PCR, endocervical samples, Iran

## Introduction

*Chlamydia trachomatis* (*C. trachomatis*) is an obligate intra-cellular pathogen and the most frequent sexually transmitted bacterium worldwide (2-5). While most of the infections occur without symptoms, asymptomatic manifestation of urogenital chlamydial infection can be observed in ~30% of the patients. In a subset of female patients, ascending genital tract infections caused disease such as salpingitis, pelvic inflammatory disease (PID), or tubal infertility and abortion (6-10). *C. trachomatis* in the cervix could be transmitted to a neonate during passage through an infected birth canal, resulting in neonatal pneumonia (11, 12).

The gold standard for diagnosis of *C. trachomatis* infection has traditionally been a culture of swab from the endocervix in women or the urethra in men (11). However, the methodological challenges of culturing of this organism and difficulty of this methods led to the development of non-culture-based tests, including antigen-detection tests and nucleic acid hybridization. These techniques however, fail to detect substantial proportion of infections. Newer tests are accordingly required to develop that could amplify and detect *C. trachomatis*-specific DNA or RNA sequences, giving rise to more sensitivity than the first generation non-culture-based tests (12-16). In many studies, target gene for NAATs were cryptic plasmid. Some studies give evidence or suggest that the plasmid-free

variants are present in clinical samples. Thus, the infections caused by plasmid-free variants will be undetected if the plasmid is used as target gene (14) .

Targets for amplification have included both the cryptic plasmid (7, 8, 10, 18, 19, 22-24, 27) and chromosomal genes, including those for the major outer membrane protein (MOMP) (2, 11, 23, 25, 33), *gyrA*, phospholipase D endonuclease superfamily gene (PRPHA). An object of this study is to propose using of unique sequences in the genome of *C. trachomatis*(17).

## Materials and Methods

**Study site and population:** This was a cross-sectional study conducted over the period from, May 2012 to February 2013. During a 10- month- period, 240 endocervical samples were taken from married women referred to Qazvin Kowsar Gynecology Hospital. Endocervical swabs were collected from women referred to Qazvin Kowsar Gynecology Hospital by training midwife. The specimens were tested for *C trachomatis* by PCR-based assay for *phagene*.

**Sample collection** Briefly, cervical mucus was removed prior to insertion of a Dacron swab into the endocervical canal. The swab was immersed in 1 ml of phosphate buffered saline (PBS) transport medium. All PBS media were maintained at 4°C during specimen collection and then aliquoted into DNase and RNase free microtubes and frozen at -80°C within 4h of collection until DNA extraction(18).

### **DNA Extraction:**

DNA was extracted from endocervical samples using boiling technique and DNG PLUS . The concentration of DNA samples was determined as micrograms per milliliter based on A260 values and adjusted to 1 µl/ml prior to PCR amplification.

### **Detection of *C. trachomatis* by PCR:**

PCR assay was performed for detection of *C. trachomatis* by *phagene* .The primers were F-(5'TCTTTTAAACCTCCGGAACCCACTT 3')  
R-(5'GGATGGCATCGCATAGCATTCTTTG3')

PCR amplification was performed in a final volume of 20 µl reaction mixture containing 1µl of each primers, 10 µl master mix sayber green (Bioneer), 6 µl distilled water, 2 µl DNA samples. Sequenced *C.*

*trachomatis* DNA was used as positive control and distilled water was used as negative control.

DNA amplification was carried out with the following thermal cycling profile: for *pha* gene 10 min of initial denaturation at 95°C followed by 35 cycles of amplification. Each cycle consisted of denaturation at 95°C for 15 S, annealing at 60°C for 30 S, and extension at 72°C for 30 S.

The PCR products were analyzed by 1.5% agarose gel electrophoresis and the product size estimated using a 100-bp DNA ladder.

**Statistical analyses:** Data Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, V. 19.0; SPSS Inc, Chicago, IL, USA). Data were summarized by frequencies and percentages for categorical variables and normally distributed data, means and standard deviation. Results were analyzed by chi-square tests. A level of  $P < 0.05$  was considered to indicate statistical significance.

## Results

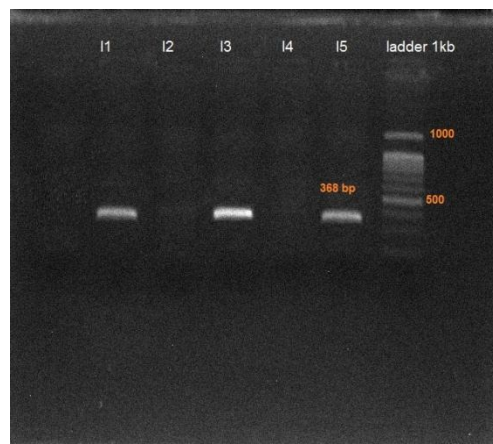
The study population was 240 married women (184 (76.7%) symptomatic and 56 (23.3%) asymptomatic cases). The mean age of cases was  $37.1 \pm 0.9$  years. In the period of this study, 240 married women enrolled including: 18 (7.5%) infertility, 58 (24.16%) abortion, vaginosis 126 (52.5%) (sign and symptom include vaginal discharge, vagina and irritation and dysuria), 13 (5.4%) premature birth, 15 (6.25%) low birth weight below 2.5 kg. Out of the 240 endocervical swab samples were tested 20 (8.3%) positive for *Chlamydia trachomatis* by PCR. Of the 56 asymptomatic women, 6 (10.7%) had chlamydia infection while among the 184 symptomatic cases 14 (7.6%) according to the PCR results.

Among symptomatic women with *Chlamydia* infection, infertility were 0 (0%), abortion were 3 (5.1%), vaginosis were 9 (7.1%), premature birth were 1 (7.7%), low birth weight were 1 (7.7%).

The  $\chi^2$  test was not show a significant relationship between positive test result and vaginosis ( $P= 0.76$ ), abortion ( $P= 0.55$ ), premature birth ( $P=1.0$ ), low birth weight below 2.5kg ( $P=1.0$ ) and infertility ( $P= 0.36$ ),

**Table 1: Frequency of chlamidial infection among married women according to symptoms**

Chracteristic	PCR Positive n(%)	PCR Negative n(%)	Total n(%)	P value
<b>Sympomatic</b>				
Infertility	0(0%)	18(100%)	18(100%)	0.36
Abortion	3(5.1%)	55(94.9%)	58(100%)	0.55
Vaginosis	9(7.1%)	117(92.9%)	126(100%)	076
Premature birth	1(7.7%)	12(92.3%)	13(100%)	1.0
Low birth weight below 2.5kg	1(7.7%)	12(92.3%)	13(100%)	1.0
<b>Asympomatic</b>				
Healthy women	6(10.7%)	50(89.3%)	56(100%)	



**Figure 1-PCR amplification of *C. trachomatis* DNA Positive clinical sample, 1 kb plus DNA ladder L1L5Negativeclinical sample,L3, L6 control Positive, L2,L4Positiveclinical sample**

## Discussion

*Chlamydia trachomatis* is one of the most frequent causes of sexually transmitted diseases(2, 3). It is a common cause of urethritis and cervicitis. Some reported sequelae include PID, abortion, tubal factor infertility, epididymitis, proctitis and reactive arthritis (6-10) .

The majority of chlamydia-infected individuals are asymptomatic, and remain unnoticed and untreated. Infected women may be at risk of gynaecological complications (e.g. PID and tubal infertility), and determine the reservoir for onward transmission in the population(19).

In the current study, the prevalence of chlamydial infection among 240 women attending Kowsar Gynecology Hospital in Qazvin was 8.3%. This rate in asymptomatic *C. trachomatis* infections cases were 6 (10.7%), while 14 (7.6%) of symptomatic cases were infected. The  $\chi^2$  test was not show a significant relationship between positive test result and vaginosis ( $P=0.76$ ), abortion ( $P=0.55$ ), premature birth ( $P=1.0$ ), low birth weight below 2.5kg ( $P=1.0$ ) and infertility ( $P=0.36$ ).

Torrone et al, reported the prevalence of *Chlamydia trachomatis* genital infection among persons aged 14–39 years in United States, during 2007–2012 was 1.7% . Overall prevalence of chlamydial infection among persons aged 14–39 years was similar over the three National Health and Nutrition Examination Survey (NHANES) cycles combined for this analysis: 2007–2008: 1.6% (CI = 1.1%–2.2%); 2009–2010: 1.7% (CI = 1.2%–2.1%); and 2011–2012: 1.9% (CI = 1.5%–2.2%). *Chlamydia* is the most commonly reported nationally notifiable disease, with over 1.4 million infections reported in 2012 . However, case reports likely underestimate the burden of disease because most infections are asymptomatic and are neither diagnosed nor reported. The 2007–2012 NHANES indicate that an estimated 1.8 million persons aged 14–39 years in the United States have a genital chlamydial infection. These results showed that increasing chlamydial infection during 2007–2012 in USA(20).

In a number of studies from other countries, showed the different rate of frequency of *Chlamydia trachomatis* endocervical infection in women.

Marcone et al, reported the prevalence of *C. trachomatis* infection of women in Rome. The mean prevalence of *C. trachomatis* endocervical infection during 2000 to 2009 was 5.2%. The results of this study showed that the high rate of sexually transmitted infections (STI)(21).

Al-Thani et al, reported prevalence of *C. trachomatis* infection among 377 (37.9% Qataris, 62.1% non-Qataris) healthy women attending primary healthcare centers

in Qatar. The specimens were tested for *C. trachomatis* by PCR-based assay. Prevalence of *C. trachomatis* infection was 5.3% among Qatari women and 5.5% among non-Qatari women. Similar to our study, the high prevalence may reflect, in part, the limited access to and use of chlamydia screening and management(10).

Araujo, R., et al in central Brazil reported the overall prevalence of *C. trachomatis* infection by PCR was 19.6%(22).

Cuffini et al, reported prevalence of genital *Chlamydia trachomatis* Infection in Asymptomatic women in Argentina was 13.7% (23).

The results showed higher prevalence of *C. trachomatis* infection among Latin American women than our study.

**conclusion:** The present study shows that *C. trachomatis* endocervical infection could be present in symptomatic (7.6% ) as well as asymptomatic women (10.7%). The majority of chlamydia-infected individuals are asymptomatic, and remain unnoticed and untreated. Infected women may be at risk of gynaecological complications (e.g. PID and tubal infertility), and determine the reservoir for onward transmission in the population. Strategies for the control of infection and prevention of its complications are only partially effective (safer sex campaigns) or not yet available (vaccine). Screening programmes have been introduced as an additional strategy for early detection and treatment of infected cases. Cost-effectiveness of screening is largely determined by the rates of complications prevented. Evidence on the impact of screening on the prevalence of chlamydia infections at a population level is still limited, as is the impact on the prevalence of complications in screened women.

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